

NORBERT KOTHE ET AL  
BEIL WOLFF 291-KGB

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Kotthe*  
25. The method of claim 22, wherein the first fraction obtained is worked up in a known manner and therapeutically usable antithrombin III, transferrin and/or albumin are obtained.

*11*  
26. The method of claim 22, wherein the second fraction obtained is worked up in a known manner and therapeutically usable immunoglobulin, especially IgG, is obtained.

*11*  
27. The use of an immunoglobulin preparation or an antithrombin III, albumin or transferrin preparation, obtained according to the method of claim 1, for therapeutic application.

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REMARKS

*11*  
Applicant desires to begin the national stage procedure based on the amended claims 1-  
27. The amendments above eliminate multiple dependencies, and place the claims in better form  
for U.S. examination.

Early and favorable action is earnestly solicited.

Respectfully submitted,  
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NORBERT KOTHE ET AL  
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**MARK-UP SHOWING THE CHANGES MADE IN THE PREVIOUS CLAIM TO YIELD  
THE CLAIM AS AMENDED ABOVE**

3. The method of [claims 1 or 2] claim 1, wherein an ammonium sulfate gradient is used for the chromatography.

5. The method of [one of the claims 3 or 4] claim 3, wherein the high concentration of ammonium sulfate is between 0.6 and not more than 1.4 moles/L and the low ammonium concentration is between 0 and 0.4 moles/L.

6. The method of [one of the claims 3 to 5] claim 3, wherein the high concentration of ammonium sulfate buffer is 0.7 to 1 moles/L, which is lowered to 0 to 0.3 moles/L.

7. The method of [one of the claims 1 to 6] claim 1, wherein the starting solution and the chromatography phase are adjusted to the desired high salt gradient concentration at the start of the fractionation.

8. The method of [one of the claims 1 to 7] claim 1, wherein plasma, from which the clotting factors of the PPSB complex were removed by a known procedure, is used as starting material.

9. The method of [one of the claims 1 to 8] claim 1, wherein clotting factor VIII is removed from the starting material by a known procedure, and this starting material is used for the preparative fractionation.

10. The method of [one of the claims 2 to 9] claim 2, wherein polyvalent human plasma is used as a starting material.

NORBERT KOTHE ET AL  
BEIL WOLFF 291-KGB

11. The method of [one of the claims 2 to 9] claim 2, wherein selected human plasma, selected with respect to viral, bacterial or antibodies, directed against cellular antigens, is used as starting material.

12. The method of [one of the claims 1 to 11] claim 1, wherein, after the first fraction, two further fractions are obtained by means of step gradients.

14. The method of [one of the claims 1 to 13] claim 1, wherein phenyl-substituted or alkyl-substituted phases, based on copolymers of glycidyl methacrylate and ethylene glycol dimethacrylate, copolymers of polystyrene or divinylbenzene or silica, coated with dextran or polymers, are used as hydrophobic interaction phase.

16. The method of [claims 14 or 15] claim 14, wherein the high concentration of ammonium sulfate buffer is 0.8 to 1.0 moles/L and the lowered concentration of ammonium sulfate is 0.3 to 0 moles/L.

18. The method of [one of the claims 1 to 17] claim 1, wherein the first fraction obtained is worked up in a known manner and therapeutically usable antithrombin III, transferrin and/or albumin are obtained.

20. The method of [one of the claims 1 to 18] claim 1, wherein the second fraction obtained is worked up in a known manner and therapeutically usable immunoglobulin, especially IgG, is obtained.

22. A recycling method for fractionating plasma or serum of [one of the claims 1 to 16] claim 1, wherein a starting solution, containing plasma or serum, is chromatographed on a

NORBERT KOTHE ET AL  
BEIL WOLFF 291-KGB

preparative scale without initial rivanol precipitation with a stepwise salt gradient with hydrophobic interaction chromatography and, by such a procedure, at least one immunoglobulin-containing fraction and one albumin-containing fraction are obtained and the permeate is then supplied from the first albumin fraction obtained continuously to the ammonium sulfate buffer reservoir with the buffer solution 1 with an ammonium sulfate concentration of the first high step, the first fraction obtained is collected continuously, the second immunoglobulin-containing-fraction is eluted by preparing a mixed buffer from the buffer solution 1 and an ammonium sulfate-free buffer solution 2 or by using only the buffer 2 with the low ammonium sulfate concentration of the 2<sup>nd</sup> step and removed continuously.

24. The method of [one of the claims 22 or 23] claim 22, wherein, after each recycling cycle, the interaction chromatography phase is treated with sodium hydroxide solution from a reservoir 3.

25. The method of [one of the claims 22 to 24] claim 22, wherein the first fraction obtained is worked up in a known manner and therapeutically usable antithrombin III, transferrin and/or albumin are obtained.

26. The method of [one of the claims 22 to 24] claim 22, wherein the second fraction obtained is worked up in a known manner and therapeutically usable immunoglobulin, especially IgG, is obtained.

27. The use of an immunoglobulin preparation or an antithrombin III, albumin or transferrin preparation, obtained according to the method of [one the claims 1 to 26] claim 1, for therapeutic application.